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Characterization of a Group B *Streptococcus* infection based on the demographics, serotypes, antimicrobial susceptibility and genotypes of selected isolates from sterile and non-sterile isolation sites in three major hospitals in Malaysia

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KEYWORDS
Group B *Streptococcus*;
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Summary
Background/purpose: The purpose of this study is to characterize GBS isolates that were collected from three major hospitals in a densely populated area of Klang Valley for their demographics, serotypes, antibiotic susceptibility patterns and genetic background.
Methods: Sixty GBS isolates from sterile and non-sterile samples in three major hospitals in the Klang Valley area of Malaysia were collected by convenience sampling

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from 2012 until March 2014. These isolates were studied for their antimicrobial susceptibilities, serotypes and genotypes. Patients' demographic data and clinical information were collected from lab request forms.
Results: Diabetes mellitus was the only underlying condition (7 patients, 23.3%); the remaining samples were from patients who were immunocompromised due to medications. Fifty-nine (98%) isolates were sensitive to penicillin, while 78.3% and 88.3% of the isolates were sensitive to erythromycin and clindamycin, respectively. Serotype Ia was the most common serotype ($n = 27$, 45%), followed by serotype III ($n = 10$, 16.7%), V ($n = 9$, 15%), VI ($n = 8$, 13.3%), VIII ($n = 2$, 3.3%) and VII ($n = 1$, 1.7%). Random Amplified Polymorphic DNA (RAPD) typing showed a diverse genetic pedigree for all isolates, including four major groups that clustered according to geographical location.
Conclusion: This preliminary study determines the prevalence of limited common serotypes and antimicrobial resistance in distinct GBS isolates. Nonetheless, the RAPD clustering pattern suggests a close genetic lineage of the GBS isolates based on their isolation sites and location of hospitals.

Introduction

Group B *Streptococcus* (GBS), also known as *Streptococcus agalactiae*, is a frequent colonizer of the vagina in pregnant women, as well as the gastrointestinal and genitourinary tract in healthy adults [1]. GBS can cause a variety of severe diseases, such as sepsis, pneumonia and meningitis, in neonates. It also promotes serious mortality in immunocompromised patients and morbidity in pregnant women and non-pregnant adults [2,3]. The Centers for Disease Control and Prevention (CDC) published guidelines for intrapartum antibiotic prophylaxis in 1996 and recommended penicillin or ampicillin as the drugs of choice for the prevention of perinatal GBS disease [4], while erythromycin and clindamycin may serve as alternative antibiotics for women who are allergic to penicillin [5]. An important factor that contributes to GBS pathogenicity is capsular polysaccharide *(cps)*. Generally, nine serotypes (Ia, Ib and II to VIII) have been recognized, and most recently, serotype IX was proposed [6]. Epidemiologically, serotype Ia, III and V are commonly associated with invasive disease in pregnant women, neonates and non-pregnant adults. However, there may be geographical and temporal variations in the prevalence pattern of the serotypes [7]. To the best of our knowledge, published reports on GBS cases in Malaysia are still limited. Therefore, the purpose of this study was to characterize GBS isolates collected from a few major hospitals in Malaysia for their demographics, serotypes, antibiotic susceptibility patterns and genetic background. Comparative analysis was conducted to reveal potential epidemiological patterns of local GBS isolates.

Methods

Bacterial isolates and patients' clinical information

This study included 60 GBS isolates collected from sterile and non-sterile sites of patients at three major hospitals located in the densely populated area of Klang Valley; Hospital Serdang and Universiti Kebangsaan Malaysia Medical Centre (UKMMC), which are the teaching and referral hospitals in Malaysia, and Hospital Kuala Lumpur (HKL), which is the largest government tertiary referral hospital located on 150 acres of prime land in the Federal Territory of Kuala Lumpur with 83 wards and 2302 beds. The samples were collected at convenience to include viable isolates with available patients' information from 2012 until 2014; 10 isolates each from sterile and non-sterile sites were randomly targeted from each hospital. Of the 60 GBS isolates, 28 cases were in the year 2013, 21 cases in 2014 and 11 cases in 2012. The present study reviewed information on demographic data, site of isolation, associated underlying conditions and clinical presentation. All isolates were identified by standard laboratory methods and kept at $-70\,^{\circ}C$ for long-term preservation and at $4\,^{\circ}C$ for short-term storage.

Antimicrobial susceptibility testing

Antimicrobial susceptibility testing (AST) was performed by disc diffusion (Kirby—Bauer) method according to CLSI guidelines [8]. The panel of antibiotics included a beta-lactam (penicillin), a macrolide (erythromycin), a lincosamide (clindamycin), a phenicol (chloramphenicol) and tetracycline. Etest® strip (Liofilchem®Rosetodegli Abruzzi (Te), Italy) was utilized with GBS isolates to determine the Minimal Inhibitory Concentration (MIC) of isolates that had high or intermediate resistance to penicillin, erythromycin and clindamycin based on the disc diffusion test. *Streptococcus pneumoniae* ATCC 49619 was used for quality control. Double disc diffusion (D test) was also applied to check for inducible clindamycin resistance in GBS isolates resistant to erythromycin but susceptible to clindamycin.

Serotyping

Ten serotype (Ia, Ib and II to IX) reagents were tested against GBS isolates by conventional serotyping (CS) via Latex Agglutination Kit (Statens Serum Institute, SSI, Sweden). Isolates that failed to form any agglutination with any serotype reagents were labeled as non-typeable (NT). Molecular serotyping (MS) by multiplex PCR was also performed on all isolates using a series of primers according to Imperi et al. [9]. DNA was extracted using GeneAll Exgene Cell SV Mini genomic extraction kit (GeneAll, South Korea) according to the manufacturer's instructions.

RAPD-based genotyping

DNA from GBS isolates was subjected to Random Amplified Polymorphic DNA (RAPD) assay reaction to determine the genetic backgrounds of the isolates. Polymerase chain reaction (PCR) cycling conditions were applied as described by Duremdez et al. [10] with P2 [10] and GBS-2 primers [11] using a DNA thermocycler (Biometra-TRIO Thermoblock). DNA electrophoretic patterns were analyzed using BioNumerics gel analysis software Version 7.0 (Applied Maths, Belgium) on the basis of the presence or absence of DNA bands. RAPD dendrograms based on each and a composite of all primers were then generated by the software using the average coefficient UPGMA cluster analysis.

Statistical analysis

Chi-Square test of independence was used to analyze the tabulations among the serotype, antimicrobial patterns, hospital source of isolation and genotypic properties using GraphPad Prism software Version 5 (GraphPad Software, USA) with $p < 0.05$ as the significance level.

Results

Distribution of bacterial isolates

Table 1 shows the tabulation of the GBS isolates in relation to patient demographic and isolation sites. Of the sixty GBS-associated patients, there were 22 (36.7%) males and 38 (63.3%) females. A majority was in the 21—40-year-old age group ($n = 28$, 46.7%), followed by the 41—60-year-old ($n = 18$, 30%) and 61—80-year-old age group ($n = 8$, 13.3%). In the 21—40-year-old age group, the majority of GBS were isolated from non-sterile sites ($n = 24$, 85.7%), which comprised high vaginal swab ($n = 22$, 92%) and placenta swab ($n = 2$, 8%), while those in the 41—80-year-old age group were isolated from

Table 1 Distribution of the GBS isolates in relation to the age, isolation site, sex and ethnicity of patients.

Age (y)	Isolation site[a]		Sex		Ethnicity			
	Sterile ($n = 30$)	Non-sterile ($n = 30$)	Male ($n = 22$)	Female ($n = 38$)	Malay ($n = 49$)	Chinese ($n = 2$)	Indian ($n = 7$)	Punjabi ($n = 2$)
0—10 ($n = 6$)	6	0	4	2	6	0	0	0
11—20 ($n = 0$)	0	0	0	0	0	0	0	0
21—30 ($n = 16$)	1	15	0	16	15	1	0	0
31—40 ($n = 12$)	3	9	3	9	11	0	1	0
41—50 ($n = 7$)	5	2	4	3	5	0	2	0
51—60 ($n = 11$)	7	4	5	6	4	1	4	2
61—70 ($n = 4$)	4	0	4	0	4	0	0	0
71—80 ($n = 4$)	4	0	2	2	4	0	0	0

y = 10 year interval.
[a] Sterile site: Blood, Tissue; Non-sterile site: High vaginal swab, Urine, Placenta swab.

Table 2

CS based-serotype	MS-based serotype										Total (CS)
	Ia	Ib	II	III	IV	V	VI	VII	VIII	IX	
Ia	15										15
Ib		0									0
II			3								3
III				7							7
IV					0						0
V						6					6
VI							5				5
VII								1			1
VIII									1		1
IX										0	0
NT	12	0	0	3	0	3	3	0	1	0	22[a]
Total (MS)	27	0	3	10	0	9	8	1	2	0	60

CS, conventional serotyping (latex); MS, molecular serotyping (multiplex PCR).
NT — non-typeable serotype.
[a] All 22 NT isolates based on CS were successfully typed by MS.

sterile sites ($n = 20$, 76.9%), which consisted of blood ($n = 14$, 70%) and tissue ($n = 6$, 30%). Among the 30 isolates from sterile sites, the associated infections observed included sepsis found in the blood samples of 8 patients (26.7%), followed by pneumonia ($n = 4$, 13.3%), tissue abscess, meningitis ($n = 3$, 10% each), and tuberculosis ($n = 1$, 3.3%). In addition, 4 samples (13.3%) were of unidentifiable unknown condition. Meanwhile, of the isolates from non-sterile samples, the majority ($n = 27$, 90%) were associated with vaginal and placental infections, followed by urinary tract infections ($n = 2$, 6.7%) and tissue abscess ($n = 1$, 3.3%). Diabetes mellitus was the only underlying condition present in the isolates ($n = 7$, 23.3%) from sterile sites, and the rest of the isolates were from patients immunocompromised due to medications. Fifty-six patients (93.3%) were of the Malay and Indian ethnic groups, and the remaining patients were Chinese and Punjabi ($n = 2$, 3.3% for each ethnic group).

Antimicrobial susceptibility testing

Disc diffusion tests showed that almost all of the isolates were sensitive to penicillin ($n = 59$, 98%) with only one isolate recorded as having intermediate resistance to penicillin (isolate B14 14). On the other hand, most of the isolates were resistant to tetracycline ($n = 54$, 90%). Erythromycin resistance was identified in 13 isolates (22%), whereas seven isolates (12%) showed resistance to clindamycin. There was only one isolate from the 15 isolates that was resistant (intermediate) to erythromycin and also showed inducible clindamycin resistance in the D test. No significant association was found between the erythromycin- and clindamycin-resistant isolates in relation to isolation sites, ethnicity and age of patients ($p > 0.05$).

Serotyping

Among the 60 isolates, 38 isolates (63.3%) were successfully serotyped using a latex agglutination kit, while 22 isolates (36.7%) were non-typeable (NT). Nevertheless, the later NT isolates were successfully typed by MS. Furthermore, MS also gave results consistent with the earlier serological data on the 38 isolates. Taking CS and MS together, the most common serotype in this study was Ia ($n = 27$, 45%), followed by III ($n = 10$, 16.7%), V ($n = 9$, 15%), VI ($n = 8$, 13.3%), VIII ($n = 2$, 3.3%) and VII ($n = 1$, 1.7%). Serotype Ib, IV and IX were not found in the present study. (Table 2).

The distribution of various serotypes according to resistance isolates is shown in Table 3. Only serotype Ia, III and V isolates were found to be resistant to erythromycin and clindamycin. Of these three serotypes, only serotype III was significantly distributed among the isolates resistant to

Table 3 Distribution of serotypes Ia, III and V in relation to erythromycin and clindamycin resistance of the GBS isolates.

Serotype	Erythromycin $n = 13$	Clindamycin $n = 7$
Ia ($n = 6$)	5 (38)	1 (14)
III ($n = 9$)	5 (38)*	4 (57)*
V ($n = 5$)	3 (24)	2 (29)

% is indicated in (brackets).
* Indicate a significant association ($p < 0.05$).

Table 4 Distribution of serotypes in relation to sterile and non-sterile isolation sites of the GBS isolates.

Serotype	Sterile $n = 30$	Non-sterile $n = 30$
Ia ($n = 27$)	12 (41)	15 (53)
II ($n = 3$)	0 (0)	3 (11)
III ($n = 10$)	7 (24)	3 (11)
V ($n = 9$)	4 (14)	5 (18)
VI ($n = 8$)	6 (21)	2 (7)

See Table 1 for specimen from sterile and non-sterile isolation sites.
% is indicated in (brackets).

erythromycin ($p < 0.05$) and clindamycin ($p < 0.01$). No significant association is observed for serotypes Ia and V with antibiotic resistance in GBS (to erythromycin and clindamycin) ($p > 0.05$).

The association of serotypes according to isolation sites is illustrated in Table 4. Serotype VII and VIII were detected in a limited number of isolates and were thus excluded. No significant association was found between serotypes and isolation sites in the present study ($p > 0.05$).

RAPD analysis

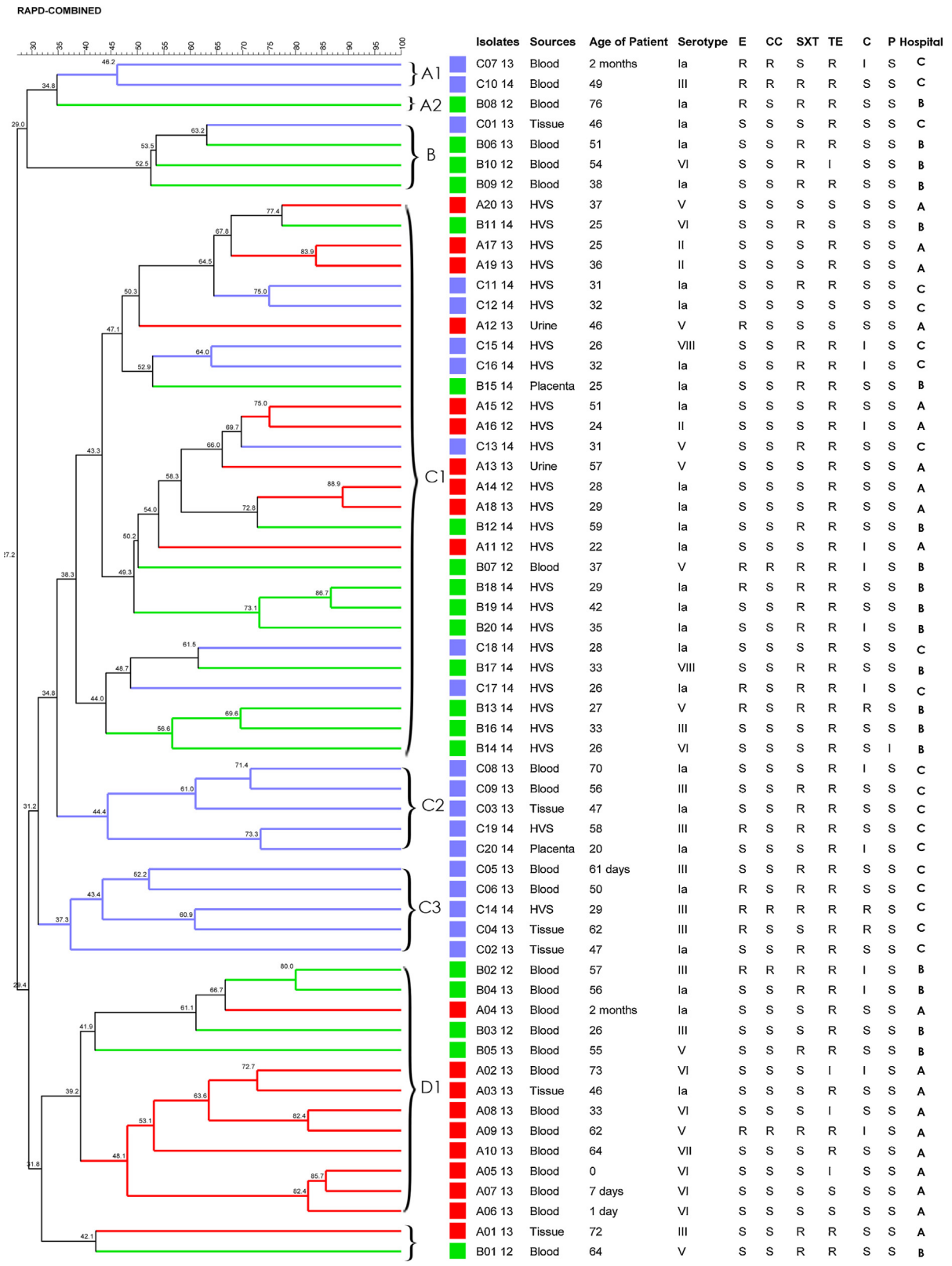
Composite analysis of the dendrogrammatic DNA profiles generated by the two primers was used in this study (Fig. 1). The analysis showed that the isolates are genetically diverse. Looking at the overall pattern, isolates were grouped in four major clusters, named cluster A, B, C and D at only 31% similarity. At 37% similarity, cluster A was further divided into sub-cluster A1 and A2, cluster C was further clustered in three sub-clusters named C1, C2 and C3 and cluster D was further divided into sub-clusters D1 and D2. Interestingly, isolates were positioned closely according to their isolation sites and the hospital's location. The majority of the isolates from Hospital Serdang were positioned closely in cluster D, whereas most of the isolates from UKMMC were positioned closely in cluster B and from HKL in cluster C. Most of the sterile isolates were positioned closely in cluster A, B and D, whereas many of non-sterile isolates were positioned closely in cluster C.

The distribution of isolates according to their respective RAPD dendrogram cluster in relation to their isolation site, hospital, resistance isolates and serotype are illustrated in Table 5. Cluster A is mainly associated with GBS isolates resistant to erythromycin ($p < 0.005$) and clindamycin ($p < 0.01$), while cluster B is frequently distributed with isolates from invasive sites ($p < 0.05$). Cluster C is more common with non-sterile isolates compared with isolates from sterile sites ($p < 0.005$), and this cluster is also frequently associated with isolates from HKL ($p < 0.05$) and serotype

Table 5 Distribution of isolation site, hospital, antimicrobial resistance and serotype of the GBS isolates in relation to RAPD dendrogram clusters.

	Cluster			
	A ($n = 3$)	B ($n = 4$)	C ($n = 38$)	D (n = 15)
Isolation site				
Sterile ($n = 30$)	3 (10)	4 (13)*	8 (27)	15 (50)***
Non sterile ($n = 30$)	–	–	30 (100)***	–
Hospital				
H. Serdang ($n = 20$)	–	–	10 (50)	10 (50)***
UKMMC ($n = 20$)	1 (5)	3 (15)	11 (55)	5 (25)
HKL ($n = 20$)	2 (10)	1 (5)	17 (85)*	–
Resistance isolates				
Erythromycin ($n = 13$)	3 (23)***	–	8 (62)	2 (15)
Clindamycin ($n = 7$)	2 (28.6)**	–	3 (42.8)	2 (28.6)
Serotype				
Ia ($n = 27$)	2 (7.4)	3 (11.1)	19 (70.4)	3 (11.1)*
II ($n = 3$)	–	–	3 (100)	
III ($n = 10$)	1 (10)	–	6 (60)	3 (30)
V ($n = 9$)	–	–	6 (67)	3 (33)
VI ($n = 8$)	–	1 (12.5)	2 (25)*	5 (62.5)***

% is indicated in (brackets).
* $p < 0.05$.
** $p < 0.01$.
*** $p < 0.005$.



Isolates	Sources	Age of Patient	Serotype	E	CC	SXT	TE	C	P	Hospital
C07 13	Blood	2 months	Ia	R	R	S	R	I	S	C
C10 14	Blood	49	III	R	R	R	R	S	S	C
B08 12	Blood	76	Ia	R	S	R	R	S	S	B
C01 13	Tissue	46	Ia	S	S	S	R	S	S	C
B06 13	Blood	51	Ia	S	S	R	R	S	S	B
B10 12	Blood	54	VI	S	S	R	I	S	S	B
B09 12	Blood	38	Ia	S	S	R	R	S	S	B
A20 13	HVS	37	V	S	S	S	S	S	S	A
B11 14	HVS	25	VI	S	S	R	S	S	S	B
A17 13	HVS	25	II	S	S	S	R	S	S	A
A19 13	HVS	36	II	S	S	S	R	S	S	A
C11 14	HVS	31	Ia	S	S	R	R	S	S	C
C12 14	HVS	32	Ia	S	S	S	S	S	S	C
A12 13	Urine	46	V	R	S	S	S	S	S	A
C15 14	HVS	26	VIII	S	S	R	R	I	S	C
C16 14	HVS	32	Ia	S	S	R	R	I	S	C
B15 14	Placenta	25	Ia	S	S	R	R	S	S	B
A15 12	HVS	51	Ia	S	S	S	R	S	S	A
A16 12	HVS	24	II	S	S	S	R	I	S	A
C13 14	HVS	31	V	S	S	R	R	S	S	C
A13 13	Urine	57	V	S	S	S	R	S	S	A
A14 12	HVS	28	Ia	S	S	S	R	S	S	A
A18 13	HVS	29	Ia	S	S	S	R	S	S	A
B12 14	HVS	59	Ia	S	S	R	R	S	S	B
A11 12	HVS	22	Ia	S	S	S	R	I	S	A
B07 12	Blood	37	V	R	R	R	R	I	S	B
B18 14	HVS	29	Ia	R	S	R	R	S	S	B
B19 14	HVS	42	Ia	S	S	R	R	S	S	B
B20 14	HVS	35	Ia	S	S	R	R	I	S	B
C18 14	HVS	28	Ia	S	S	S	R	S	S	C
B17 14	HVS	33	VIII	S	S	R	R	S	S	B
C17 14	HVS	26	Ia	R	S	R	R	I	S	C
B13 14	HVS	27	V	R	S	R	R	R	S	B
B16 14	HVS	33	III	S	S	S	R	S	S	B
B14 14	HVS	26	VI	S	S	S	R	S	I	B
C08 13	Blood	70	Ia	S	S	S	R	I	S	C
C09 13	Blood	56	III	S	S	R	R	S	S	C
C03 13	Tissue	47	Ia	S	S	R	R	S	S	C
C19 14	HVS	58	III	R	S	R	R	S	S	C
C20 14	Placenta	20	Ia	S	S	S	R	I	S	C
C05 13	Blood	61 days	III	S	S	R	R	S	S	C
C06 13	Blood	50	Ia	R	S	R	R	S	S	C
C14 14	HVS	29	III	R	R	R	R	R	S	C
C04 13	Tissue	62	III	R	S	S	R	R	S	C
C02 13	Tissue	47	Ia	S	S	R	R	S	S	C
B02 12	Blood	57	III	R	R	R	R	I	S	B
B04 13	Blood	56	Ia	S	S	R	R	I	S	B
A04 13	Blood	2 months	Ia	S	S	S	R	S	S	A
B03 12	Blood	26	III	S	S	S	R	S	S	B
B05 13	Blood	55	V	S	S	R	R	S	S	B
A02 13	Blood	73	VI	S	S	S	I	I	S	A
A03 13	Tissue	46	Ia	S	S	S	R	S	S	A
A08 13	Blood	33	VI	S	S	S	I	S	S	A
A09 13	Blood	62	V	R	R	R	R	I	S	A
A10 13	Blood	64	VII	S	S	S	R	S	S	A
A05 13	Blood	0	VI	S	S	S	I	S	S	A
A07 13	Blood	7 days	VI	S	S	S	S	S	S	A
A06 13	Blood	1 day	VI	S	S	S	S	S	S	A
A01 13	Tissue	72	III	S	S	R	R	S	S	A
B01 12	Blood	64	V	S	S	R	R	S	S	B

Figure 1 RAPD dendrogram of the GBS isolates based on composite analysis in correlation with the hospital location, type of sources, age of patient, serotype and antimicrobial susceptibility; E, Erythromycin; CC, Clindamycin; TE, Tetracycline; C, Chloramphenicol and P, Penicillin. S, Susceptible; I, Intermediate and R, Resistant. A: Hospital Serdang, B: UKMMC, C: HKL.

VI ($p < 0.05$). Cluster D also showed a significance distribution of sterile isolates ($p < 0.005$), and this cluster is also commonly distributed with isolates from Hospital Serdang ($p < 0.005$), serotype Ia ($p < 0.05$) and serotype VI ($p < 0.005$).

Discussion

The results of the antimicrobial susceptibility tests suggest that penicillin may still be a drug of choice for treating GBS due to its use as an intrapartum antibiotic prophylactic, although one high vaginal swab isolate from UKMMC (B14 14) showed intermediate resistance to penicillin. Although no isolates were recorded as resistant to penicillin in this study, cases of reduced susceptibility to penicillin have been reported elsewhere in various regions in the world. Thus, the implementation of network national surveillance should be held to monitor the emergence of penicillin resistance among GBS isolates [12—14]. On the other hand, the resistance rates toward erythromycin and clindamycin were higher in isolates from sterile sites compared with those from non-sterile sites. Nevertheless, statistical analysis showed no significant difference for resistant rates against erythromycin and clindamycin in those from non-sterile sites ($p > 0.05$).

Molecular approaches, such as multiplex PCR, allow some non-typeable GBS isolates to be successfully serotyped by identifying surface proteins [1,15—17]. A high occurrence of serotype Ia in this study is consistent with another local study [18] that reported that Ia was the common serotype comprising 22.2% among 45 isolates. Generally, serotype Ia, III and V are commonly present in pregnant women and neonates, while type V was the common serotype among non-pregnant adults [19]. There is also a high occurrence of serotype III, as well as V, in cases of resistance to erythromycin and clindamycin. This potential association involving serotype III and V is of concern as the two serotypes are usually associated with invasive strains [20].

In another study in Germany, the most prevalent serotype among the identified erythromycin-resistant isolates was serotype V (37%), followed by III (27%) [21]. In Korea, the most common serotype among erythromycin (lincosamide)-resistant isolates was also from serotype V (76%) [22].

RAPD analysis revealed that the isolates from a similar hospital and similar isolation sites are more related in their genetic lineage as reflected in the dendrogrammatic clustering of the isolates. To a certain extent, there may be an association between serotype and antimicrobial activities in some of the genetic clusters, but most of the respective isolates were distantly clustered, suggesting a long lineage of common origin. Overall, this preliminary study suggests that the occurrence of genetically distinct GBS in this local setting with serotype Ia is the most common regardless of the isolation site and location, whereas most of erythromycin- and clindamycin-resistant isolates are significantly distributed among serotype III.

Conclusion

In short, MS is a promising approach in determining serotypes. This preliminary study suggests that serotype III was significantly more common in isolates that were resistant to erythromycin and clindamycin, although serotype Ia was the most common among all of the GBS isolates regardless of the isolation site and location. The isolates were largely distinct, but RAPD analysis segregated the GBS isolates according to geographical area and isolation sites. This may imply that isolates collected from different hospitals could have their own respective genetic lineage and are possibly genetically adapted, either with colonizing or invasive capabilities. Studies involving more isolates are necessary to confirm the observed phenotypic and molecular features.

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Competing interests

None declared.

Ethical approval

Not required.

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